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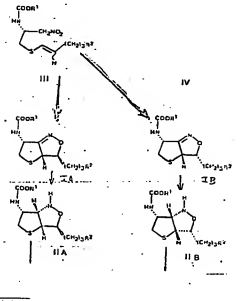
(54) **Novel process for the manufacture of thiophene derivatives suited for the manufacture of biotin, process for the manufacture of biotin and novel intermediates in this process.**

(57) The process starts from L-cystine and proceeds via various novel intermediates, particularly the isoxazolines and isoxazolidines of the formulae I and II.



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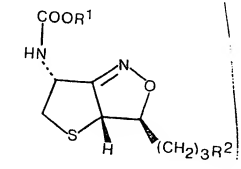
Novel process for the manufacture of thiophene derivatives suited for the manufacture of biotin, process for the manufacture of biotin and novel intermediates in this process.

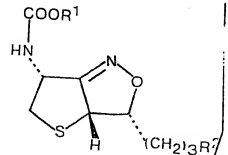
The present invention is concerned with a novel process for the manufacture of thiophene derivatives and of biotin, respectively.

The novel thiophene derivatives provided by the present invention have the general formulae

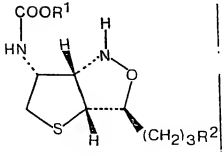


IA

and

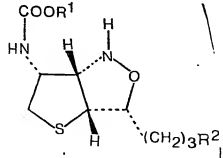


IB



IIA

and



IIB

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wherein R^1 is lower alkyl or aryl, R^2 is methyl or $-CH_2OR^3$ and R^3 is lower alkyl, aryl or aryl(lower)alkyl.

These thiophene derivatives are valuable intermediates in the synthesis of biotin.

Optically active d-biotin, also known as Vitamin H, is a natural product found in kidney, liver, egg yolk, milk and yeast. The compound can be utilized to prevent the symptoms of egg white injury in experimental animals and is used medicinally to treat various dermatitides.

Biotin has been prepared synthetically by Harris et al. (Science, 97, 447, (1943) and Baker et al. (J. Org. Chem., 12, 167, (1947) and the first commercial synthesis resulted from the work of Goldberg and Sternbach as described in U.S. Patents No. 2,489,235 and No. 2,489,236. The prior art processes for the production of optically active d-biotin proceeded via racemic intermediates and thus formed racemic mixtures of biotin. To produce the desired d-enantiomer, the resulting biotin had to be resolved by costly and time consuming techniques which led to a decrease in yield of the desired product.

The present invention now concerns a novel process for the manufacture of novel optically active intermediates for optically active d-biotin and for optically active d-biotin itself from optically active L-cystine.

As used herein, alkyl connotes straight or branched chain saturated aliphatic hydrocarbon groups containing 1 to 20 carbon atoms. "Lower alkyl" means alkyl groups having from 1 to 7 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl and the like."Lower alkoxy"means alkoxy groups having from 1 to 7 carbon atoms such as methoxy, ethoxy, isopropoxy and the like. "Lower alkylene" denotes alkylene groups of 2-6 carbon atoms such as ethy-

lene, propylene, butylene and the like. "Lower alkanol" connotes alkanols having 1-7 carbon atoms such as methanol, propanol and the like. "Lower alkylenedioxy" denotes a moiety derived by the condensation of the hydroxy group of a 1,2 or 1,3 diol with a carbonyl function. The alkylene group of lower alkylenedioxy has 2 to 5 carbon atoms. Typical lower alkylenedioxy groups are ethylenedioxy, 1,2 propylenedioxy, 2,4 butylenedioxy and 2,3 pentylenedioxy.

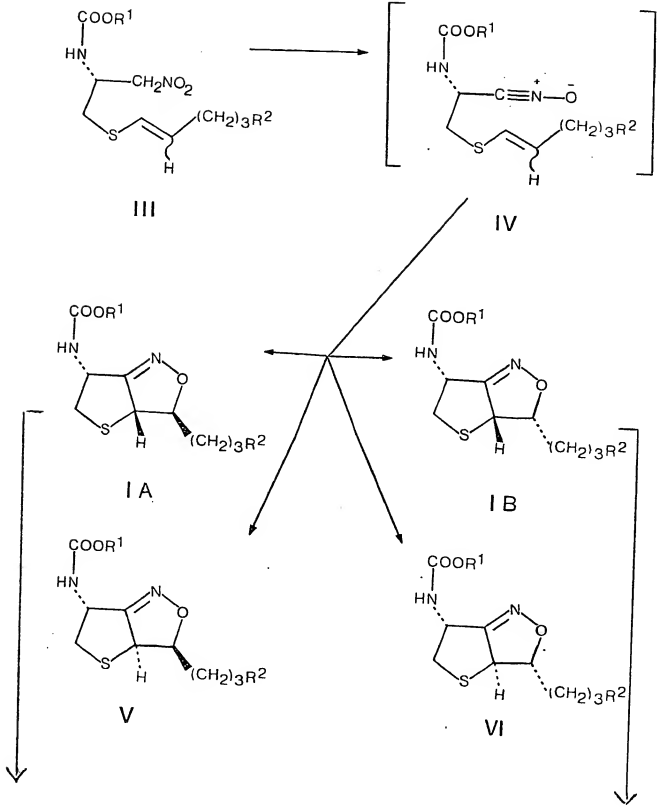
"Aryl" denotes mononuclear aromatic hydrocarbon groups such as phenyl and the like, which can be unsubstituted or substituted in one or more positions with halogen, nitrogen, lower alkylenedioxy, lower alkyl or lower alkoxy as well as polynuclear aryl groups such as naphthyl, anthryl, phenanthryl, azulyl and the like, which can be unsubstituted or substituted with one or more of the aforementioned substituents.

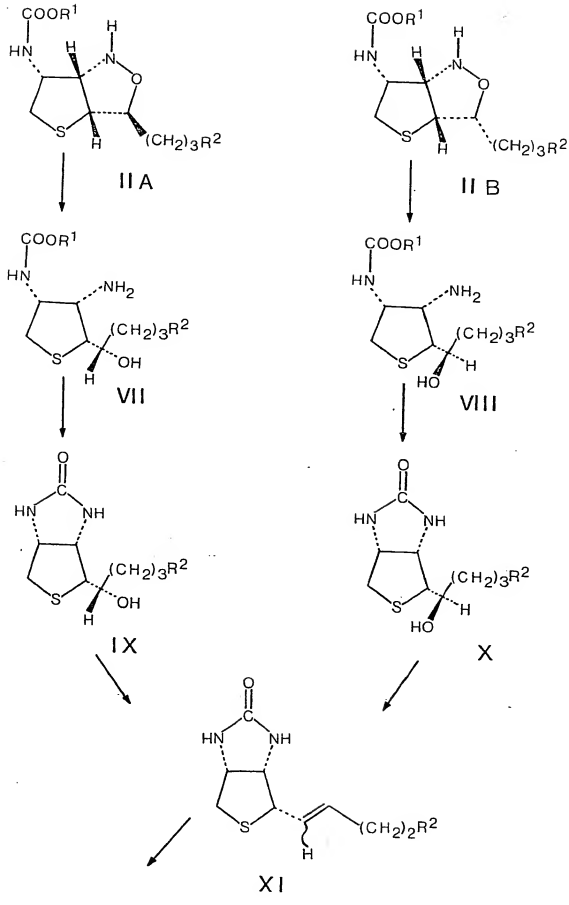
"Arylalkyl" connotes a group comprising alkyl and aryl moieties as defined hereinbefore. "Aryl(lower)alkyl" defines a group comprising lower alkyl and aryl moieties as defined hereinbefore, particularly benzyl and α-lower alkyl substituted benzyls, e.g. cumyl. "Halogen" denotes chlorine, bromine and iodine. Alkali metals include lithium, sodium, potassium and rubidium. Alkaline earth metals include beryllium, magnesium, calcium and strontium.

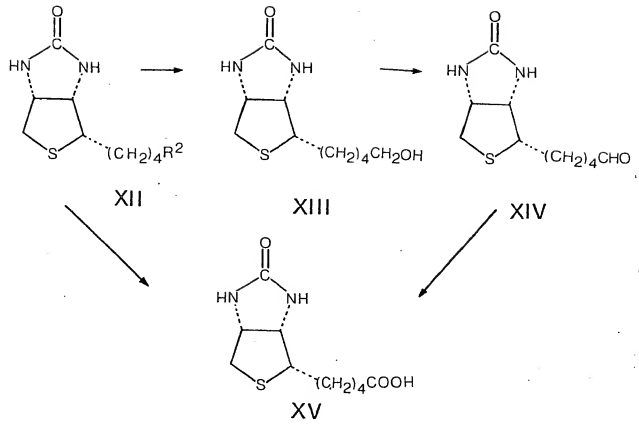
In the pictorial representations of the compounds of this application, a solid tapering line (▶) indicates a substituent which is in the ß-orientation (above the plane of the molecule) and a dashed line (---) indicates a substituent which is in the α-orientation (below the plane of the molecule). A wavy line (∿∿) indicates a Z and E mixture of the represented compound.

In accordance with the present invention, the novel isoxazolines and d-biotin, respectively, can be prepared from according to the following reaction scheme:

Scheme I




COOR1
HN
H
N
O
S
H
(CH2)3R2
II A
II B
NH2
OH
VII
VIII
O
C
NH
IX
X
(CH2)2R2
XI


HN
NH
O
C
S
(CH2)4R2
XII
(CH2)4CH2OH
XIII
(CH2)4CHO
XIV
(CH2)4COOH
XV

In this reaction scheme the various radicals have the following meanings:

R^1 is lower alkyl or aryl; R^2 is methyl or $-CH_2OR^3$ and R^3 is lower alkyl, aryl or aryl(lower) alkyl.

In accordance with the present invention, the nitro-olefins of formula III are dehydrated to the isomeric isoxazolines of formulae IA, IB, V and VI via a transient intermediate of formula IV. The conversion of the nitro-olefin III to the isooxazolines of formulae IA, IB, V and VI proceeds by an intramolecular 1,3 dipolar addition of the transient intermediate IV to its double bond and results in the formation of a 5,5-bicyclic ring system. This conversion is carried out by treating compound III with a dehydrating agent. Suitable dehydrating agents include alkyl or aryl isocyanates such as methyl isocyanate, isopropyl isocyanate, phenyl isocyanate and the like. The reaction proceeds in an inert organic solvent such as an aliphatic hydrocarbon, e.g. pentane or hexane, an aromatic hydrocarbon e.g. benzene or toluene or an ether e.g. dioxane or tetrahydrofuran. Although not critical, the temperature generally ranges from about 10°C to about 80°C. However, the reaction preferably proceeds at about room temperature.

The isomeric isoxazolines of formulae IA, IB, V and VI can be separated from each other by conventional separation techniques. For example, compounds IA and IB can be separated from compounds V and VI by open column chromatography on silica. Compounds IA and IB can then be separated from each other preferably by high pressure liquid chromatography on silica.

According to the present invention, compounds IA and IB can be utilized to form d-biotin of formula XV.

More particularly, the isoxazolinesIA and IB are first reduced to the isoxazolidines of formulae IIA and IIB, respectively, by any hydride reducing agent which is capable of selectively reducing an isoxazoline without attacking a urethane group. Suitable reducing agents include diborane and a dialkyl aluminium hydride, e.g. diisobutyl aluminium hydride. This reduction is carried out in an inert solvent such as an aliphatic hydrocarbon, e.g. isopropane, an aromatic hydrocarbon, e.g. benzene, or an ether e.g. diethylether. When utilizing diborane, the conversion of compounds IA and IB to compounds IIA and IIB, respectively, generally proceeds between about $-30^{o}C$ and about $30^{o}C$ and preferably at about $0^{o}C$. When utilizing a dialkyl aluminium hydride, the reaction generally proceeds at from about $-80^{o}C$ to about $-20^{o}C$ and preferably at from about $-60^{o}C$ to about $-50^{o}C$.

Isoxazolidines IIA and IIB are then converted to the amino alcohols of formulae VII and VIII, respectively, by catalytic hydrogenation in a suitable solvent. Typical catalysts include platinum, palladium on charcoal and Raney nickel. Any organic solvent utilized in conventional catalytic hydrogenation may be employed. Suitable solvents include organic alcohols such as methanol, isopropanol and hexanol as well as acetic acid. Although not critical, the temperature of the noted reaction generally ranges from about $0^{o}C$ to about $50^{o}C$. Room temperature is preferred.

The amino alcohols VII and VIII, respectively, can then be cyclized to the imidazolones of formulae IX and X, respectively, by treatment with a base. Suitable bases include alkali metal and alkaline earth metal hydroxides and alkoxides, e.g. sodium hydroxide, barium hydroxide and potassium alkoxide, as well as tertiary amines, e.g. pyridine and triethyl amine. Although not necessary, any conventional organic solvent or aqueous mixture thereof

may be employed in the reaction. Typical solvents include dioxane and diethyl ether. In the conversion of compounds VII and VIII, respectively, to compounds IX and X, respectively, temperature can range from about 50°C to about 150°C. When barium hydroxide is utilized with a dioxane-water mixture, reflux temperature is preferred.

The imidazolones IX and X, respectively, can be converted to an olefinic mixture of Z and E geometric isomers of the formula XI by dehydration. For example, compounds IX and X, respectively, can be reacted in the presence of paratoluene sulfonic acid in an inert aromatic solvent, such as toluene, to produce the geometric isomers of formula XI. The temperature of the reaction generally ranges from about 60°C to about 130°C. When toluene is the solvent, a temperature of about 100°C is preferred.

The compounds of formula XI are novel compounds and also form part of the present invention.

The compounds of formula XI can be converted to the known compounds of formula XII via catalytic hydrogenation. Any conventional catalytic hydrogenation technique may be employed. Typical catalysts include palladium on charcoal, palladium on barium sulfate, platinum and Raney nickel. Any conventional solvent utilized in catalytic hydrogenation may be employed. Suitable solvents include organic alcohols, e.g. methanol, propanol and hexanol, acetic acid and water mixtures thereof. Although not critical, the temperature and pressure can range from about 5°C to about 200°C and from atmospheric pressure to about 34 atm. When palladium on charcoal is selected as the catalyst, room temperature and atmospheric pressure are preferred.

The conversion of compound XII to d-biotin may be effected in various ways depending on the R^2 substituent.

When R^2 is methyl, compound XII is directly converted to d-biotin by microbiological oxidation techniques. The microbiological oxidation technique disclosed in U.S. Patent No. 3,859,167 is preferably employed. According to this procedure, compound XII, wherein R^2 is methyl, is converted to d-biotin by treatment with the organism Corynebacterium Primorioxydans.

When R^2 is $-CH_2OR^3$ and R^3 is lower alkyl, aryl or aryl(lower)alkyl, compound XII is converted to d-biotin of formula XV via intermediates of formulae XIII and XIV.

When R^3 is lower alkyl, aryl or aryl(lower)alkyl, compound XII is converted to the alcohol XIII by conventional procedures for removing ether protecting groups. A suitable technique includes acid-catalyzed hydrolysis of compound XII. Conventional strong aqueous inorganic acids may be utilized. Typical acids include aqueous hydroiodic, hydrobromic and hydrochloric acid. The reaction generally proceeds at reflux temperature.

Alternatively, when R^3 is benzyl or α-substituted benzyl, the conversion of compound XII to compound XIII occurs by hydrogenolysis. Typical hydrogenolysis agents include platinum, palladium on calcium carbonate, palladium on barium sulfate and Raney nickel. For example, the reaction proceeds in the presence of hydroxylic organic solvents, e.g. organic alcohols such as methanol or isopropanol and at a temperature ranging from about 0^oC to about 80^oC. Room temperature is preferred. The pressure can vary from atmospheric pressure to 34 atm., but atmospheric pressure is preferred.

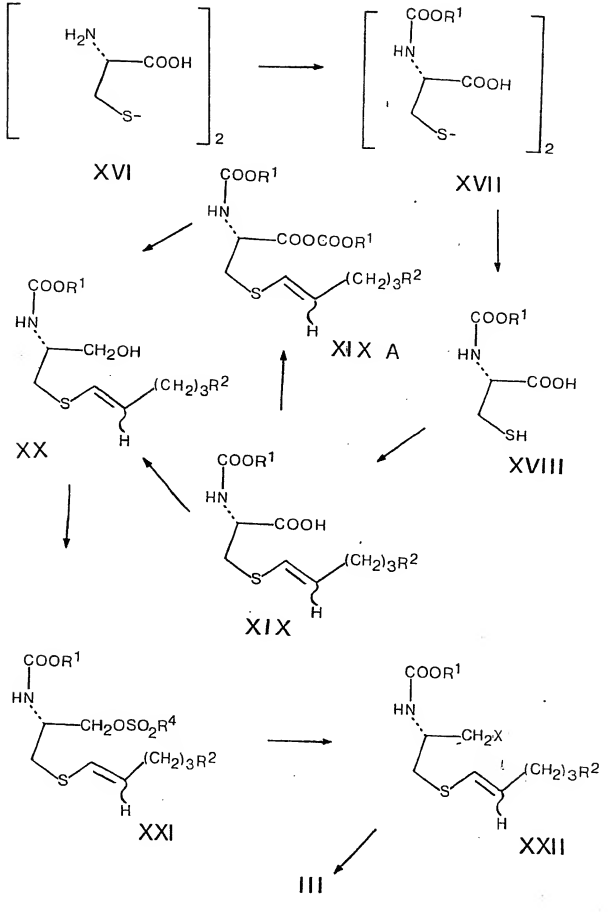
The alcohol of formula XIII is converted to an aldehyde of formula XIV by conventional procedures for selectively oxidizing an alcohol without affecting a sulfide. Typical oxidizing agents include chromium trioxide-pyri-

dine complex, pyridinium chlorochromate, and dimethyl sulfoxide. The temperature generally ranges from about -0°C to about 30°C.

The aldehyde of formula XIV is then oxidized to d-biotin by reaction with silver oxide. Preferably, the reaction proceeds in an alkali metal hydroxide-water solvent, e.g. aqueous sodium hydroxide and aqueous potassium hydroxide. Although not critical, the reaction generally proceeds from about room temperature to the boiling point of the reaction medium. Generally, a temperature of about 60°C is preferred.

In accordance with a particularly preferred embodiment of the process of the instant invention, the mixture of isomers I-A and I-B need not be separated into individual compounds to form d-biotin. Pursuant to the previously described reaction conditions and procedures, a mixture of compounds I-A and I-B can be converted to a mixture of compounds II-A and II-B, which can be converted to compounds VII and VIII, which in turn can be cyclized to a mixture of compounds IX and X, which can be followed by dehydration to compound XI. The latter compound can be converted to known compound XII, which can be converted to d-biotin by the procedures described previously.

The compounds of the above formula III are novel and can be prepared according to the following reaction scheme:


XVI
XVII
XIX A
XX
XVIII
XIX
XXI
XXII
III

In this reaction scheme, the radicals have the following meanings:

R^1 and R^2 have the meanings given previously; R^4 is lower alkyl or aryl and X is halogen.

In accordance with the above noted scheme, L-cystine of formula XVI is acylated at the nitrogen atom to form an urethane of formula XVII according to conventional techniques. More particularly, L-cystine of formula XVI is reacted with a lower alkyl haloformate or aryl haloformate, e.g. methylchloroformate, ethylchloroformate or phenylbromoformate, in basic media to form compound XVII. The base media may be inorganic or organic. Typical inorganic bases include alkali metal hydroxides, carbonates and bicarbonates, e.g. sodium hydroxide, sodium carbonate or potassium bicarbonate, and the organic bases include tertiary amines, e.g. pyridine or triethyl amine. Although not necessary, the reaction may proceed in an inert organic solvent, e.g. dioxane or tetrahydrofuran, or an aqueous mixture thereof, e.g. a mixture of water and tetrahydrofuran. The temperature is not critical but the reaction is generally carried out between about -30°C and about 30°C, preferably at about 0°C.

The urethane of formula XVII is reduced to the mercaptan of formula XVIII via any conventional means for selectively reducing a disulfide bond. A suitable technique includes reacting compound XVII with a hydride reducing agent such as an alkali metal or alkaline earth metal borohydride, e.g. sodium borohydride or magnesium borohydride, in an appropriate organic solvent, e.g. methanol, ethanol and hexanol for sodium borohydride and diethyl ether for magnesium borohydride. Although the temperature is not critical, the reaction generally proceeds from about -10°C to about 30°C, preferably at about 0°C.

In another method for converting compound XVII to compound XVIII, compound XVII is reacted with a dissolved alkali metal reducing agent, such as sodium in liquid ammonia, at a temperature of from about -80°C to about 0°C and preferably at about -40°C.

Compound XVIII is reacted with 1-hexyne or 6-lower alkoxy, 6-aryl(lower)alkoxy or 6-aryloxy substituted 1-hexynes in the presence of a radical initiator to form an olefinic carboxylic acid mixture of Z and E geometric isomers of formula XIX. Typical substituted acetylenic compounds include 6-ethoxy-1-hexyne, 6-benzyloxy-1-hexyne and 6-phenoxylhexyne. Any conventional radical initiator such as benzoyl peroxide, 2',2'-bisazo-(2-methylpropionitrile), di-t-butyl peroxide or dicumene may be utilized in this reaction. The conversion of compound XVIII to compound XIX is carried out in an inert organic solvent such as an ether, e.g. diethyl ether, dioxane or tetrahydrofuran, or an aromatic hydrocarbon, e.g. benzene or toluene. The reaction is carried out at a temperature sufficient to decompose the radical initiator and generally ranges from about 50°C to about 150°C depending upon the initiator utilized.

The carboxylic acid of formula XIX is reduced to an alcohol mixture of Z and E geometric isomers of formula XX by any conventional means for selectively reducing a carboxylic acid to an alcohol without affecting an urethane moiety. An acceptable reducing agent for this selective reduction is diborane in an inert organic solvent such as an aliphatic hydrocarbon, e.g. pentane or hexane, an aromatic hydrocarbon, e.g. benzene or toluene, or an ether, e.g. diethyl ether or dioxane. The reaction temperature generally ranges from about -30°C to about 30°C. Preferably the reaction proceeds at about 0°C.

Compound XIX also can be converted to compound XX

via intermediate XIX-A. Compound XIX first is reacted with a lower alkyl haloformate or aryl haloformate to form anhydride XIX-A which is then converted via a hydride reducing agent to compound XX. Typical lower alkyl haloformates or aryl haloformates include methyl chloroformate, ethyl chloroformate and phenyl bromoformate. Suitable hydride reducing agents include complex borohydrides of alkali metals and alkaline earth metals, e.g. sodium borohydride, potassium borohydride or magnesium borohydride. The formation of the anhydride XIX-A and the reduction thereof occurs in an inert organic solvent, e.g. hexane, benzene or diethyl ether, and at a temperature of from about -20°C to about 30°C, preferably at about 0°C.

The alcohol of formula XX ist then reacted with a lower alkyl halosulfonate or aryl halosulfonate, e.g. methyl sulfonyl chloride or paratoluene sulfonyl bromide, in the presence of a tertiary amine such as pyridine or triethyl amine, to form a sulfonate mixture of Z and E geometric isomers of formula XXI. Although not necessary, the reaction is preferably carried out in an inert organic solvent such as an ether, e.g. diethyl ether or tetrahydrofuran, or an aromatic hydrocarbon, e.g. benzene or toluene. Although temperature is not critical, the reaction proceeds generally between about -20°C and about 60°C. A temperature of about 0°C is preferred.

Compound XXI is treated with an alkali metal halide in a suitable solvent to form a halide mixture of Z and E geometric isomers of formula XXII. Among the preferred alkali metal halogenates are sodium iodide, sodium bromide and potassium bromide. Typical solvents include dialkyl ketones such as acetone, dialkyl sulfoxides such as dimethyl sulfoxide and dialkyl amides such as dimethylformamide. The reaction is generally carried out between about room temperature to about 100°C and preferably at about 50°C to about 70°C.

The compound of formula XXII is converted to a nitro olefin mixture of Z and E geometric isomers of formula III by reacting compound XXII with an alkali metal nitrite. Among the preferred alkali metal nitrites are sodium nitrite, potassium nitrite and lithium nitrite. The reaction proceeds in an organic solvent having a high dielectric constant. Suitable solvents include dimethyl formamide, hexamethyl phosphoramide and dimethyl sulfoxide. The formation of compound III from compound XXII generally occurs at a temperature between about 10°C and about 50°C and preferably at about room temperature.

Although the above discussions are directed particularly to the conversion of optically active L-cystine of formula XVI to the optically active isoxazolines and isoxazolidines of formulae I-A, I-B, II-A and II-B and to optically active d-biotin of formula XV, respectively, the present invention is not to be construed as limited thereto. For example, the process of the present invention may be utilized to convert the racemate of L-cystine to the racemate of d-biotin via racemates of the previously described intermediates.

More particularly, the racemate of L-cystine of formula XVI can be acylated at the nitrogen to the racemate of urethane XVII, which can be reduced to the racemate of mercaptan XVIII, followed by conversion to the racemate of olefinic carboxylic acid XIX. The latter racemate can then be reduced to a racemate of alcohol XX, which can be converted to the racemate of sulfonate XXI, which in turn can be converted to the racemate of compound XXII and subsequently to the racemate of nitro olefin III. The racemate of compound III can be dehydrated to the racemates of isomeric isoxazolines I-A, I-B, V and VI. These isomers can then be separated by conventional means. The racemate of compounds I-A and/or I-B can then be converted to the racemate of isoxazolidine II-A and/or II-B,

which in turn can then be converted to the racemate of the hydroxy amine VII and/or VIII, followed by cyclization to the racemate of imidazolone IX and/or X. The latter racemate is then dehydrated to the racemate of olefin XI, which in turn is catalytically hydrogenated to the racemate of compound XII. When R^2 of racemate XII is methyl, said racemate can be directly converted to the racemate of biotin XV by the process disclosed in U.S. Patent No. 3,859,167. When R^2 is $-CH_2OR^3$ racemate XII is converted into the racemate of compound XIII which in turn is selectively oxidized to the racemate of aldehyde XIV, which then can be converted to the racemate of biotin of formula XV. If desired, the racemate of formula XV can be converted to d-biotin by conventional resolution techniques and procedures.

If desired, any of the racemates formed by the aforementioned procedure can be converted to its desired optically active enantiomer by conventional resolution procedures. For example, the selected racemate can be reacted with a conventional resolving agent and the reaction products can be separated by known techniques such as crystallization and chromatography. The optically active enantiomer can then be converted to optically active d-biotin by the process previously described.

If desired, any of the Z and E geometric isomer mixtures of formulas XIX-XXII; III and XI can be converted to their corresponding Z and E components via conventional chromatographic techniques. Suitable methods are open column chromatography on silica and high pressure liquid chromatography.

The following examples are illustrative of the instant invention. Unless otherwise indicated, all temperatures are in degrees Centigrade (^oC) and the ether is diethyl ether.

Example 1

L-N-(methoxycarbonyl)-cystine

A solution of 13.8 g (0.13 mol) of sodium carbonate, dissolved in 180 ml of water was mixed with 120 ml of a 10 o/o solution of sodium bicarbonate in water, to which 14.4 g (0.06 mol) of L-(-)-cystine were added. The resulting suspension was cooled at 0°C and treated dropwise with 12.3 g (10.1 ml, 0.13 mol) of methylchloroformate. After addition, the reaction mixture was allowed to come to room temperature and vigorously stirred for 4 hours. It was then cooled again at 0°C and adjusted to pH 2 with 5N hydrochloric acid, allowed to come to room temperature, saturated with sodium chloride and extracted with 3 x 150 ml of ethyl acetate. The combined organic layers were dried and evaporated in vacuo to give 19.16 g (92 o/o crude yield) of L-N-(methoxycarbonyl)-cystine as a thick pale yellow oil.

Example 2

L-N-(methoxycarbonyl)-cysteine

A solution of 19.6 g (0.055 mol) of crude L-N-(methoxycarbonyl)-cystine in 200 ml of dry liquid ammonia was treated at -60°C to -70°C with metallic sodium added portionwise in small pieces and waiting, after each addition, so that the initially blue solution turned colorless again. Altogether, 4.8 g (0.209 mol) of sodium were used. When the blue color persisted, a few crystals of ammonium chloride were added until the color was discharged and then the ammonia was allowed to evaporate. The residue was treated with 100 ml of a saturated ammonium chloride solution, the pH was adjusted to 2 with 5N hydrochloric acid, and the solution extracted with 3 x 150 ml of ethyl acetate. The organic layers were combined, dried and evaporated

in vacuo to give 18.0 g (91.4 o/o crude yield) of L-N-(methoxycarbonyl)-cysteine as a thick pale yellow oil.

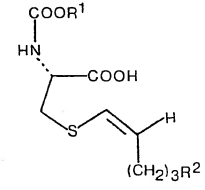
Example 3

(Z and E)-S-(hexen-1-yl)-N-(methoxycarbonyl)-cysteine

A solution of 10.74 g (0.06 mol) of L-N-(methoxycarbonyl)-cysteine, 7.15 g (10 ml, 0.087 mol) of 1-hexyne and 500 mg of 2,2'-bisazo-(2-methylpropionitrile) in 20 ml of dioxane was heated at 85°C for 10 hours, cooled at room temperature and diluted with 200 ml of ether and extracted with 3 x 100 ml of a 2N solution of sodium hydroxide. The combined alkaline solutions were washed with 3 x 100 ml of ether and adjusted to pH 2 with 3N hydrochloric acid (at 0°C). The resulting mixture was extracted with 3 x 150 ml of ethyl acetate. The combined organic extracts were washed with 3 x 50 ml of brine, dried and evaporated to give 14.0 g (89 o/o crude yield) of (Z and E)-S-(hexen-1-yl)-N-(methoxycarbonyl)-cysteine as a pale, yellow oil.

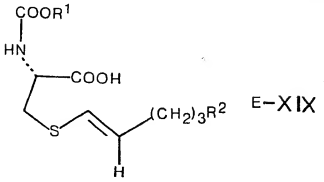
The above mixture of Z and E geometric isomers can be separated into its Z and E components by preparative high pressure liquid chromatography.

The Z component generically can be expressed as:



Z-XIX

and the E component generically can be described as:



E-XIX

wherein R^1 is lower alkyl or aryl; R^2 is methyl or $-CH_2OR^3$; and R^3 is lower alkyl, aryl or aryl(lower)alkyl.

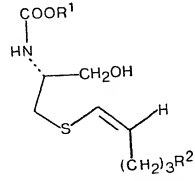
Example 4

(Z and E)-2R-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)-amino]propanol

To a solution of 32.0 g (0.123 mol) of (Z and E)-S-(hexen-1-yl)-N-(methoxycarbonyl)-cysteine in 250 ml of freshly distilled dry tetrahydrofuran cooled at 0°C, 13.6 g (0.134 mol) of triethylamine and 12.8 g (0.135 mol) of methylchloroformate were subsequently added dropwise. After addition, the reaction mixture was stirred for 2 1/2 hours, filtered and slowly added to a suspension of 22.7 g (0.600 mol) of sodium borohydride in 100 ml of water at 0°C. The resulting mixture was allowed to come to room temperature, stirred for 3 hours, cooled again at 0°C and treated with 65 ml of 5N hydrochloric acid, which were added dropwise. Finally, the solution was extracted with 3 x 150 ml of ethyl acetate and the combined organic layers were washed with 3 x 50 ml of a 2N potassium bicarbonate solution, followed by 3 x 50 ml of brine, dried and evaporated in vacuo to give 25.8 g (87 o/o crude yield) of (Z and E)-2R-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)amino]propanol, as an almost colorless thick oil.

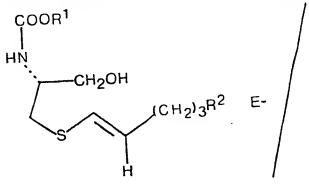
The above mixture of Z and E geometric isomers can be separated into its Z and E components by preparative high pressure liquid chromatography by elution with a mixture of n-hexane and ethyl acetate (1:1 parts by volume).

The Z component generically can be expressed as:



Z-XX

and the E component generically can be described as:



E-

wherein R^1 is lower alkyl or aryl; R^2 is methyl or $-CH_2OR^3$ and R^3 is lower alkyl, aryl or aryl(lower)alkyl.

Example 5

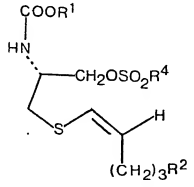
(Z and E)-2R-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)-amino]-1-[(methylsulfonyl)oxy]propane

A solution of 13.1 g (0.050 mol) of (Z and e)-2R-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)amino]propanol in 60 ml of anhydrous pyridine was treated dropwise at 0°C with 8.6 g (0.075 mol) of methanesulfonylchloride. After addition, the reaction mixture was further stirred

at 0°C for 4 hours. Then, it was diluted with 10 ml of water, stirred for 30 minutes, adjusted to pH 2 with 2N hydrochloric acid and extracted with 3x100 ml of ethyl acetate. The combined organic phases were washed with water, 2N potassium bicarbonate solution, brine, dried and evaporated in vacuo to give 16.2 g (99 o/o crude yield) of (Z and E)-2R-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)amino]-1-[(methylsulfonyl)oxy]propane as a thick, pale yellow oil.

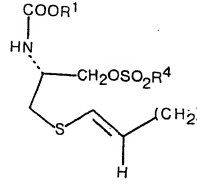
The above mixture of Z and E geometric isomers can be separated into its Z and E components by preparative high pressure liquid chromatography.

The Z component generically can be expressed as:



Z-XXI

and the E component generically can be described as:



E-XXI

wherein R^1 and R^4 are each lower alkyl or aryl; R^2 is

methyl or $-CH_2OR^3$ and R^3 is lower alkyl, aryl or aryl(lower)alkyl.

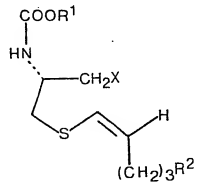
Example 6

(Z and E)-2S-3-(1-hexen-1-yl-thio)-1-iodo-2-[(methoxycarbonyl)amino]propane

A mixture of 16.2 g (0.050 mol) of (Z and E)-2R-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)amino]-1-[(methylsulfonyl)oxy]propane, 22.5 g (0.150 mol) of sodium iodide and 350 ml of acetone was refluxed for 5 hours. After cooling, the solvent was evaporated in vacuo, the residue treated with 100 ml of water and extracted with 3x100 ml of ethyl acetate. The combined organic phases were washed with 2N sodium thiosulfate solution, water, brine and evaporated in vacuo to give 16.1 g (90.7 o/o crude yield) of (Z and E)-2S-3-(1-hexen-1-yl-thio)-1-iodo-2-[(methoxycarbonyl)amino]propane, as a thick, yellow-brown oil.

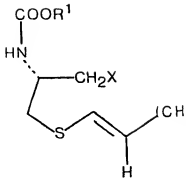
The above mixture of Z and E geometric isomers can be separated into its Z and E components by preparative high pressure liquid chromatography.

The Z component generically can be expressed as:



Z-XXII

and the E component generically can be described as:



E-XXII

wherein R^1 is lower alkyl or aryl; R^2 is methyl or $-CH_2OR^3$; R^3 is lower alkyl, aryl or aryl(lower)alkyl; and X is halide.

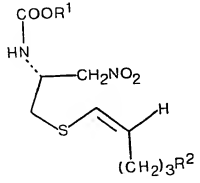
Example 7

(Z and E)-2R-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)-amino]-1-nitropropane

A solution of 6.8 g (0.019 mol) of (Z and E)-2S-3-(1-hexen-1-yl-thio)-1-iodo-2-[(methoxycarbonyl)amino]-propane, 2.5 g (0,042 mol) of urea, 2.4 g (0.019 mol) of phloroglucinol and 2.9 g (0.042 mol) of sodium nitrite in 100 ml of dimethylformamide was stirred at room temperature under nitrogen, for 30 hours. The reaction mixture was then treated with 100 ml of water, extracted with 3x100 ml of ether and the combined organic layers were washed subsequently with water, 2N sodium thiosulfate solution, water, 2N potassium bicarbonate and brine. Evaporation in vacuo gave 5.39 g of a yellow residue which were purified on a silica gel column (100 g), eluted with an ethyl acetate-hexane mixture (2:3 parts by volume) to give 3.1 g (59 o/o yield) of pure (Z and E)-2R-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)amino]-1-nitropropane as a light yellow powder. Crystallization from methylene chloride-hexane gave 2.9 g (55 o/o) of crystalline (Z and E)-2R-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)-amino]-1-nitropropane, m.p. 75-76°C.

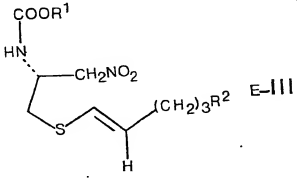
The above mixture Z and E geometric isomers can be separated into its Z and E components by preparative high pressure liquid chromatography.

The Z component generically can be expressed as:



Z-III

and the E component generically can be described as:



E-III

wherein R^1 is lower alkyl or aryl; R^2 is methyl or $-CH_2OR^3$; and R^3 is lower alkyl, aryl or aryl(lower)alkyl.

Example 8

[3S-(3ß,3aß,6α)], [(3R-(3α,3aß,6α)], [3S-(3ß,3aα,6α)] and [(3R-(3α,3aα,6α)]-3-butyl-3,3a,5,6-tetrahydrothieno-[3,2-c]isoxazol-6-yl carbamic acid methyl ester

A mixture of 1.00 g (0.0036 mol) of (Z and E)-2R-3-(1-hexen-1-yl-thio)-2-[(methoxycarbonyl)amino]-1-nitropropane, and 1.29 g (0.0109 mol) phenylisocyanate in 30 ml of anhydrous benzene, to which a few drops of triethylamine were added, was stirred at room temperature, under argon, for 40 hours. The reaction mixture was then treated with 5 ml of water, stirred at room temperature for one hour, diluted with 100 ml of benzene and the organic phase washed subsequently with water and brine, dried and evaporated to give 0.980 g of a light brown residue. This was applied on a 200 g silica gel column, eluted with a 1:2 parts by volume mixture of ethyl acetate-hexane to give 0.859 g (92.4 o/o yield) of a mixture of isoxazolines [3S-(3ß,3aß,6α)], [(3R-(3α,3aß,6α)], [3S-(3ß,3aα,6α)] and [(3R-(3α,3aα,6α)]-3-butyl-3,3a,5,6-tetrahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester. The components of the mixture were separated by high pressure chromatography, eluted with a mixture of hexane-ethyl acetate (5:1 parts by volume) to give the following isomers: 0.111 mg of pure [(3S-(3ß,3aß,6α)]-3-butyl-3,3a,5,6-tetrahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester. (Crystallization from hexane-methylene chloride afforded white crystals, m.p. 83-85°C); 0.149 mg of pure [3R-(3α,3aß,6α)]-3-butyl-3,3a,5,6-tetrahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester. (Crystallization from hexane-methylene chloride gave white crystals, m.p. 110-113°C); 0.119 mg of pure [3S-(3ß,3aα,6α)]-3-butyl-3,3a,5,6-tetrahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester. (Crystallization from methylene chloride hexane afforded white crystals, m.p. 92-94°C); and 0.122 mg of pure [3R-

(3α,3aα,6α)]-3-butyl-3,3a,5,6-tetrahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester. (Crystallization from methylene chloride-hexane gave white crystals, m.p. 102-104°C).

Example 9

[3S-(3ß,6α)cis]-3-butylhexahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester and [3R-(3α,6α)cis]-3-butylhexahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester

To a solution of 1.4 g (5.4 mmol) of the isomeric isoxazolines [3S-(3ß,3aß,6α)] and [3R-(3α,3aß,6α)]-3-butyl-3,3a,5,6-tetrahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester (approx. 1:1 mixture) in 60 ml of anhydrous toluene, kept under argon at -78°C, 18 ml (27.0 mmol) of a 1.5 molar diisobutylaluminum hydride solution in toluene were added dropwise. After addition, the reaction mixture was stirred at -78°C for one additional hour. After this time, 5 ml of methanol were added dropwise, the solution was allowed to come to room temperature and was mixed with 100 ml of a 2N aqueous Rochelle salt solution. The organic layer was separated and the aqueous phase further extracted with 3x50 ml of ethyl acetate. The combined organic phases were washed with brine, dried and evaporated in vacuo to give 1.4 g of a crude mixture of [3S-(3ß,6α)cis]-3-butylhexahydrothieno-[3,2-c]isoxazol-6-yl carbamic acid methyl ester and [3R-(3α,6α)cis]-3-butylhexahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester. The components of this mixture were separated on a 250 g silica gel column, using ethyl acetate-hexane (1:1 parts by volume) as eluent to give 0.380 g of pure [3S-(3ß,6α)cis]-3-butylhexahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester and 0.280 g of pure [3R,(3α,6α)cis]-3-butylhexahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester. Crystal-

lization of [3S-(3ß,6α)cis]-3-butylhexahydrothieno[3,2-c]-isoxazol-6-yl carbamic acid methyl ester from hexane-methylene chloride gave white crystals, m.p. 143-144°C. Crystallization of [3R-(3α,6α)cis]-3-butylhexahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester from methylene chloride-hexane afforded white crystals, m.p. 147-149°C.

Example 10

[2R-(2R*)-(2α,3α,4α)]-3-amino-α-butyl-tetrahydro-4-[(methoxycarbonyl)amino]thiophene-2-methanol

A mixture of 0.175 g (0.672 mmol) of [3S-(3ß,6α)-cis]-3-butylhexahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester and 0.050 g of 10 o/o palladium on charcoal in 15 ml of a 1:1 parts by volume mixture of acetic acid and water was hydrogenated at room temperature and under atmospheric pressure for 24 hours. The resulting solution was then neutralized by careful addition of 1N potassium bicarbonate solution and, after addition of 50 ml of ethyl acetate, filtered through Celite^{R} and washed with 5x50 ml of ethyl acetate. The separated and combined organic layers were washed with 3x20 ml of brine, dried and evaporated in vacuo to give 0.164 g (93 o/o yield) of crude [2R-(2R*)-(2α,3α,4α)]-3-amino-α-butyl-tetrahydro-4-[(methoxycarbonyl)amino]thiophene-2-methanol. This was further purified by column chromatography on 30 g of silica gel, using ethyl acetate-methanol (95:5 parts by volume) as eluent to give 0.111 g (63 o/o yield) of pure [2R-(2R*)-(2α,3α,4α)]-3-amino-α-butyl-tetrahydro-4-[(methoxycarbonyl)amino]thiophene-2-methanol. Crystallization from methylene chloride-methanol gave white crystals, m.p. 134-135°C.

Example 11

[2R-(2S*)-(2α,3α,4α)-3-amino-α-butyl-tetrahydro-4-[(methoxycarbonyl)amino]thiophene-2-methanol

A mixture of 0.120 g (0.461 mmol) of [3R-(3α,6α)cis]-3-butylhexahydrothieno[3,2-c]isoxazol-6-yl carbamic acid methyl ester and 0.050 g of 10 o/o palladium on charcoal in 15 ml of a 1:1 parts by volume mixture of acetic acid and water was hydrogenated at room temperature and under atmospheric pressure for 24 hours. The reaction mixture was then neutralized by careful addition of 1N potassium bicarbonate solution and after addition of 50 ml of ethyl acetate, filtered through Celite^{R} and washed with 5x10 ml of ethyl acetate. The separated and combined organic layers were washed with 3x20 ml of brine, dried and evaporated in vacuo to give 0.113 g (93 o/o yield) of crude [2R-(2S*)-(2α,3α,4α)-3-amino-α-butyltetrahydro-4-[(methoxycarbonyl)amino]thiophene-2-methanol. Purification by column chromatography gave 0.086 g (71 o/o) of pure [2R-(2S*)-(2α,3α,4α)-3-amino-α-butyltetrahydro-4-[(methoxycarbonyl)amino]thiophene-2-methanol. Crystallization from hexane-methylene chloride gave white crystals, m.p. 104-105°C.

Example 12

[3aS,4R,(4R*),3aß,6aß]-4-(1-hydroxypentyl)-1H-hexahydrothieno[3,4-d]imidazol-2-one

A mixture of 0.014 g (0.053 mmol) of [2R-(2R*)-(2α,-3α,4α)]-3-amino-α-butyltetrahydro-4-[(methoxycarbonyl)-amino]thiophene-2-methanol, 0.2 g of barium hydroxide, 1 ml of dioxane and 2 ml of water was refluxed under argon for 1 hour. The mixture was then acidified with 1N hydrochloric acid and extracted with 4x20 ml of ethyl acetate. The combined organic layers were washed with 2N potassium bicarbonate solution and brine, dried and evaporated in

vacuo to give 0.0115 g (93 o/o) of 3aS,4R,(4R*),3aß,6aß]-4-(1-hydroxypentyl)-1H-hexahydrothieno[3,4-d]imidazol-2-one. Crystallization from methylene chloride-hexane gave white crystals, m.p. 222-224°C.

Example 13

[3aS,4R-(4S*),3aß,6aß]-4-[1-hydroxypentyl]-1H-hexahydrothieno[3,4-d]imidazol-2-one

A mixture of 0.030 g (0.114 mmol) of [2R-(2S*)-(2α,3α,4α)]-3-amino-α-butyltetrahydro-4-[(methoxycarbonyl)-amino]thiophene-2-methanol and 0.2 g of barium hydroxide, 1.5 ml of dioxane and 2 ml of water was refluxed under argon for 1 hour. The mixture was then acidified with 1N hydrochloric acid and extracted with 4x20 ml of ethyl acetate. The combined organic layers were washed with 2N potassium bicarbonate solution and brine, dried and evaporated in vacuo to give 0.0215 g (82 o/o yield) of [3aS,4R-(4S*),3aß,6aß]-4-[1-hydroxypentyl]-1H-hexahydrothieno[3,4-d]imidazol-2-one. Crystallization from methyl chloride-hexane gave white crystals, m.p. 196-198°C.

Example 14

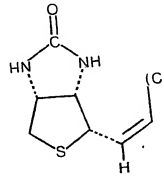
(E and Z)-[3aS,3aß,4α,6aß]-4-(1-penten-1-yl)-1H-hexahydrothieno[3,4-d]imidazol-2-one

A solution of 0.08 g (0.35 mmol) of [3aS,4R-(4R*)-3aß,6aß]-4-[1-hydroxypentyl]-1H-hexahydrothieno[3,4-d]-imidazol-2-one and 0.020 g of P-toluenesulphonic acid monohydrate in 2 ml of dry toluene was refluxed for 1 hour under argon. After cooling, 5 ml of a 2N sodium bicarbonate solution were added thereto and the resulting mixture was extracted with 3 x 20 ml of ethyl acetate. The combined organic phases were washed with brine and dried to give, after evaporation of the solvent in vacuo, 0.072 g

(98 o/o yield) of (E and Z)-[3aS,3aß,4α,6aß]-4-(1-penten-1-yl)-1H-hexahydrothieno[3,4-d]imidazol-2-one. NMR analysis showed the presence of a 5:2 ratio of E and Z geometrical isomers.

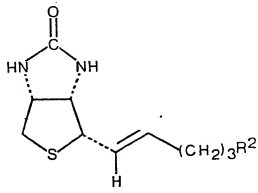
The above mixture of Z and E geometric isomers can be separated into its Z and E components by preparative high pressure liquid chromatography.

The Z component generically can be expressed as:



Z-XI

and the E component generically can be described as:



wherein R^2 is methyl or $-CH_2OR^3$; and R^3 is lower alkyl, aryl or aryl(lower)alkyl.

Example 15

(E and Z)-[3aS,3aß,4α,6aß]-4-(1-hexen-1-yl)-1H-hexahydrothieno[3,4-d]imidazol-2-one

A solution of 0.10 g (0.43 mmol) of [3aS,4R-(4S*)-3aß,6aß]-4-[1-hydroxypentyl]-1H-hexahydrothieno[3,4-d]-imidazol-2-one and 0.020 g of p-toluenesulfonic acic monohydrate in 2 ml of dry toluene was refluxed under argon for 1 hour. After cooling, 5 ml of a 2N sodium bicarbonate solution were added thereto and the resulting mixture was extracted with 3 x 20 ml of ethyl acetate. The combined organic phases were washed with brine and dried to give after evaporation of the solvent in vacuo 0.09 g (92 o/o yield) of (E and Z)-[3aS,3aß,4α,6aß]-4-(1-hexen-1-yl)-1H-hexahydrothieno[3,4-d]imidazol-2-one.

The above mixture of Z and E geometric isomers can be separated into its Z and E components by preparative high pressure liquid chromatography and have the formulae as given in Example 14.

Example 16

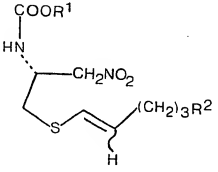
[3aS,3aß,4α,6aß]-hexahydro-4-pentyl-1H-thieno[3,4-d]imidazol-2-one

A mixture of 0.026 g (0.122 mmol) of (E and Z)-[3aS,3aß,4α,6aß]-4-(1-hexen-1-yl)-1H-hexahydrothieno[3,4-d]-imidazol-2-one, 8 ml of a 1:1 parts by volume mixture of acetic acid and water and 50 mg of 10 o/o palladium on charcoal was hydrogenated at room temperature and under atmospheric pressure overnight. It was then neutralized by careful addition of 2N potassium bicarbonate solution and, after addition of 50 ml of ethyl acetate, filtered through Celite^{R} and washed with 3 x 20 ml of ethyl acetate. The separated and combined organic layers were was-

hed with brine, dried and evaporated in vacuo to give 0.024 g (92 o/o yield) of [3aS,3aß,4α,6aß]-hexahydro-4-pentyl-1H-thieno[3,4-d]-imidazol-2-one. Crystallization from ethanol-water gave white crystals, m.p. 182-183°C.

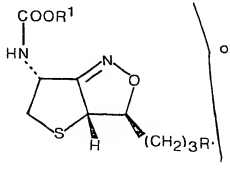
Claims

1. Process for the manufacture of novel thiophene derivatives and of biotin, respectively, characterized in that a compound of the general formula



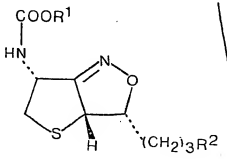
III

wherein R^1 is lower alkyl or aryl, R^2 is methyl or $-CH_2OR^3$ and R^3 is lower alkyl, aryl or aryl(lower)alkyl, is dehydrated, that, if desired, a so obtained compound of formula



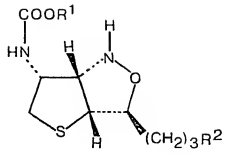
IA

or



IB

wherein R^1 is lower alkyl or aryl, R^2 is methyl or $-CH_2OR^3$ and R^3 is lower alkyl, aryl or aryl(lower)alkyl, is reduced, that, if desired, a so obtained compound of formula



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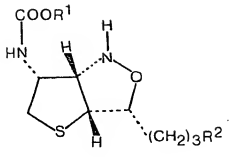
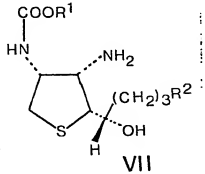


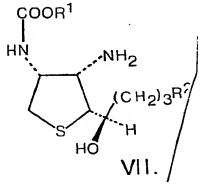
IIA IIB

wherein R^1 is lower alkyl or aryl, R^2 is methyl or $-CH_2OR^3$ and R^3 is lower alkyl, aryl or aryl(lower)alkyl, is catalytically hydrogenated, that, if desired, a so obtained compound of the general formula



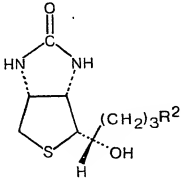
VII

or



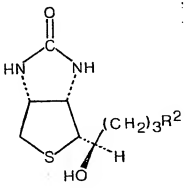
VII.

wherein R^1 and R^2 have the above meanings, is cyclized, that, if desired, a so obtained compound of the general formulae



IX

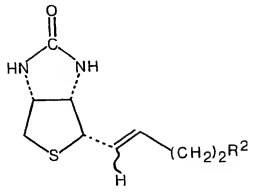
or



X

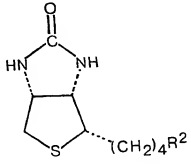
wherein R^2 has the above meanings, is dehydrated, that, if desired, a so obtained compound

of the general formula



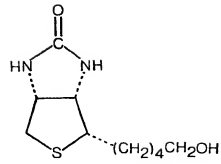
XI

wherein R^2 has the above meanings,
is catalytically hydrogenated, that, if desired, a so obtained compounds of the general formula



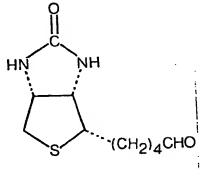
XII

wherein R^2 is methyl,
is converted into biotin by microbiological oxydation or that, if desired, from an obtained compound of formula XII, wherein R^2 is $-CH_2OR^3$ and R^3 has the above meanings, the ether protective groups are removed, that, if desired, the so obtained compound of the formula



XIII

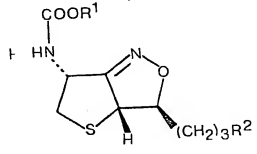
is selectively oxidized, and, that, if desired, the so obtained compound of the formula



XIV

is oxidized with silver oxide to form biotin.

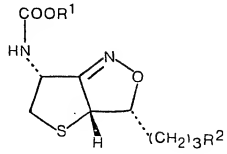
2. A compound of the general formula



I A

wherein R^1 is lower alkyl or aryl, R^2 is methyl or $-CH_2OR^3$ and R^3 is lower alkyl, aryl or aryl(lower)alkyl, or the racemate thereof.

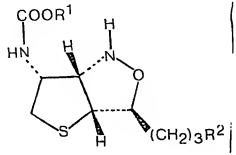
3. A compound of the general formula



IB

wherein R^2 is lower alkyl or aryl, R^2 is methyl or $-CH_2OR^3$ and R^3 is lower alkyl, aryl or aryl(lower)alkyl, or the racemate thereof.

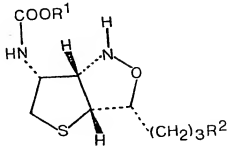
4. A compound of the general formula



IIA

wherein R^1 is lower alkyl or aryl, R^2 is methyl or $-CH_2OR^3$ and R^3 is lower alkyl, aryl or aryl(lower)alkyl, or the racemate thereof.

5. A compound of the general formula



II B

wherein R^2 is lower alkyl or aryl, R^2 is methyl or $-CH_2OR^3$ and R^3 is lower alkyl, aryl or aryl(lower)alkyl,

or the racemate thereof.

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European Patent Office

EUROPEAN SEARCH REPORT

0011777

Application number

EP [illegible] 4209

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim
P	US - A - 4 130 713 (E.G. BAGGIOLINI et al.)	

CLASSIFICATION OF THE APPLICATION (Int Cl. ⁴)

C 07 [illegible]
C 07 [illegible]
C 07 C 149/24
A 61 [illegible]
(C 07 [illegible]
C 07 [illegible]
C 07 [illegible]
(C 07 [illegible]
C 07 [illegible]
C 07 [illegible]

TECHNICAL FIELDS SEARCHED (Int Cl. ⁴)

C 07 D 495/04

CATEGORY OF CITED DOCUMENTS

X: particularly relevant
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention
E: conflicting application
D: document cited in the application
L: citation for other reasons

&: member of the same patent family, corresponding document

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	28-02-1980	ALFARO

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